REVIEW

Autologous Growth Factors: A Biological Treatment in Sports Medicine

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ABSTRACT

Autologous growth factors have been used in maxillofacial and plastic surgery since the 1990s. The use of preparations rich in growth factors has seen an increase in sports medicine is due to its potential to enhance muscle, tendon, ligament and cartilage healing, and thereby accelerating an athlete's functional return to play. The efficacy of these autologous preparations rich in growth factors has seen varying healing effects for ligament, tendon, muscle, and cartilage injuries. Current clinical evidence is in its infancy with mainly animal and retrospective human studies, but the use of preparations rich in growth factors has increased, given its favourable safety profile and efficient preparation and delivery systems. It is important for physicians to keep abreast with the latest available preparation devices and current clinical evidence looking into the effects of autologous growth factors on tendon, ligament, muscle and cartilage healing.

Keywords: blood injection therapy, conditioned plasma, ligament, muscle and cartilage healing, platelet-rich plasma, tendon,

INTRODUCTION

In the quest to accelerate musculoskeletal repair, the use of autologous biological therapies have become widespread in sports medicine. The efficacy of autologous preparations rich in growth factors (PRGF) has seen varying healing effects for ligament, tendon, muscle, and cartilage injuries. Healing is promoted by growth factors acting directly on target cells present in the injured site. Animal models have shown up-regulation in temporal expression of some growth factors and their receptors during the healing process in tendons[1,2], while healing has also been shown to take place in response to local application of growth factors[3,4]. Its efficacy in healing tendons, articular cartilage and ligaments has been shown in several studies[5–8].

WHAT ARE GROWTH FACTORS?

Growth factors are a heterogeneous group of proteins secreted by many different body tissues, which include connective tissue cells, haematopoietic stem cells, white cells, platetelets and solid organs like the liver. As growth factors have a short biological half-life and are cleared rapidly from the circulation, they exert mostly local effects. Platelets are an important component involved in haemostasis and store growth factors in alpha granules, which are activated to release these factors at the site of injury[9].

Platelets store and transport a number of important growth factors. The important growth factors include transforming growth factor Beta (TGF-B), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). The actions of these growth factors are shown in Table 1 (overleaf).

PREPARATIONS OF GROWTH FACTORS AND ADMINISTRATION

There are currently several methods of concentrating growth factors. **Platelet-rich plasma (PRP)** involves extracting the portion of plasma that contains higher concentration of platelets after

Table 1. Selected growth factors and its actions.

Growth Factor	Actions
Transforming growth factor Beta (TGF-B)[9–12]	▪ Active in most stages of tendon healing ▪ Direct stimulatory effect on collagen synthesis ▪ Stimulates osteoblasts
Platelet-derived growth factor (PDGF)[9,13]	▪ Active in early stages of healing ▪ Induces the synthesis of other growth factors ▪ Stimulates cell replication ▪ Promotes angiogenesis ▪ Promotes granulation tissue
Insulin-like growth factor (IGF)[14]	▪ Important mediator during the inflammatory stages ▪ Promotes cell proliferation
Vascular endothelial growth factor (VEGF)[1,9]	▪ Stimulates angiogenesis
Fibroblast growth factor (FGF)[9,15]	▪ Stimulates collagen proliferation ▪ Stimulates proliferation of endothelial cells ▪ Stimulates angiogenesis

centrifugation of autologous whole blood. PRP has been found to contain between 4 to 8 times the normal platelet concentrations compared with whole blood[16]. The increased concentration of platelets results in an increase in alpha-granules which store growth factors[9,16]. When delivered into damaged tissue, the platelets begin active secretion of growth factors within 10 minutes, with more than 95% of the growth factors released within the hour. The platelets remain viable for 7 days in the tissue and continue to release growth factors[18].

PRP preparations have certain steps in common. The first step is the withdrawal of the patient's peripheral blood, followed by centrifugation to yield 3 layers — the red layer (containing erthryocytes), white layer (leukocytes and inflammatory cytokines), and the yellow layer (containing plasma, platelets and growth factors)[19]. The upper most layer, the yellow layer, is extracted and used to be injected into the affected region of the body. Currently, there are many commercial systems available, for example: ACP-NS by Arthrex, GPS by Biomet and Magellan by Medtronic, to name a few[19]. These systems differ in terms of speed of centrifugation, number of centrifugations, the use of anticoagulant, the presence of leukocytes in the preparation, and the use of activators[19]. The initial blood volume withdrawn, final volume of PRP and final platelet concentration also differ between systems. Some systems do not use activators in their protocol with the reason that the preparation will be activated once in the tissues. Exogenous thrombin has been used but the argument against it is the risk of immune reactions[20]. Calcium chloride has also been used as an activator to avoid immune reactions[20]. The inclusion of leukocytes in the preparation has also been a source of controversy, as leukocytes may release matrix metalloproteinases[20] and reactive oxygen species that increase tissue damage[21]. However, whether the presence of leukocytes will have a positive or negative effect cannot be applied to all tissues and pathologies.

Delivering a higher absolute number of platelets does not necessarily make a particular system or protocol better. In bone regeneration, concentrations above 1.8×10^6 platelets/μL may have an inhibitory effect while concentrations below 3.8×10^5 platelets/μL is suboptimal[22].

Besides yielding greater concentrations of growth factors, PRP preparations also present some advantages over other methods of obtaining growth factors. Platelets have an important role in coagulation and haemostasis[19]. The alpha granules of platelets also secrete other cytokines/proteins (endostatins, platelet factor 4) that are involved in the healing process[23]. Platelets have also been found to have analgesic properties, releasing

protease-activated receptor 4 peptides[24]. Fibrin, present in PRP, allows for the creation of a scaffold for wound healing, which in turn is used for stem or primary cell migration and differentiation[19].

Autologous blood injection involves obtaining whole blood from the patient and re-injecting, normally a few millimeters, of that same blood to the site of injury. This preparation does deliver growth factor-releasing platelets, but at concentrations lower than PRP[16].

Autologous conditioned serum is another technique of yielding growth factors. The blood obtained from the patient is incubated with glass beads and spun down[25,26]. This technique yields lower concentrations of platelets and a preparation of released growth factors which is lower than PRP.

There are currently no common guidelines on the administration protocol of PRGFs, as studies vary in terms of clinical condition, chronicity or severity of condition, single or repeated injections. Studies also do not critically assess clinical outcomes when PRGFs are directly injected into injured tissue or around the tissue. The use of ultrasound-guided injections theoretically allows for accurate placement and administration of PRGFs into affected tissue, but there are no studies that have assessed this[27].

GROWTH FACTORS AND TISSUE REPAIR

Tendon Injuries

Overused tendon injuries form a significant proportion of musculoskeletal injuries. With repetitive stress injuries, tendon collagen fibres form micro tears, leading to tendinopathy. Soft tissue healing occurs in 3 phases of inflammation, proliferation and remodelling. However, often due to poor vascularisation, tendon healing tends to be slow compared with other soft tissues[28]. Furthermore, histologic examination of chronic tendon injuries reveal angiofibroblastic degeneration rather than an inflammatory response[17]. Given the inherent poor healing properties of tendon, growth factors play a significant role in healing by binding to receptors on local and circulating cells to initiate a sequence of intracellular signalling that results in the expression of proteins[29]. These proteins are important in regulating cellular chemotaxis, matrix synthesis and proliferation[29]. While there is stimulation of proliferation, excess inflammation is inhibited by suppression of macrophage proliferation and interleukin-1 production in the early stages of healing[30,31]. This creates a balance between healing and the negative effects of excessive inflammation[32].

There have been numerous studies into the effect of growth factor injection on tendons, and these have been either human or equine cell culture studies. Enhanced collagen gene expression[33], tenocyte proliferation, greater maturation in tendon callus, enhanced mobilisation of cells from the circulation to site of injection were seen[34]. Animal studies have shown growth factors are capable of increasing the strength of rotator cuff repairs[35], but researchers point out that this is achieved through the production of scar tissue, instead of the regeneration of native tissue at the tendon-bone insertion site. This alludes to the need for further work into the optimal timing and delivery method of growth factors in tissue healing.

Muscle Injuries

Injury to muscle tissue can occur from direct contact or from muscle fibre tearing due to eccentric loading when contracting[37]. This causes a range of injury from bruising to muscle tear. The conventional treatment employs modalities such as ice, electrotherapy, mobilisation, manipulation and exercise to optimise the healing process and recovery time.

Muscle healing takes place in a series of steps, which overlap — inflammation, proliferation and remodelling. The steps are regulated by the presence of growth factors and cell interactions. There is a large concentration of cytokines found in healing muscle, evidence for the role of growth factors in muscle healing. Growth factors not only enhance muscle regeneration, but also enhance muscle force[38].

Growth factors, in particular IGF, have been described in vivo to enhance muscle regeneration[39]. A study by Hammond *et al* investigated the effect of an injection protocol of PRP to the tendons of strained rat tibialis anterior muscles. There was improved recovery time to full contractile function after high repetition strains of the muscle[40].

Chondral Injuries

Research into treatment modalities for articular cartilage injuries is another growing area of interest, as the avascular cartilage has poor regeneration

capacity. The incidence of articular cartilage pathology continues to grow due to greater physical activity and sports participation, while treatment remains a challenge for sports medicine and orthopaedic specialists. An increasing concern is the subsequent progression to osteoarthritis after traumatic joint injury in sports persons. Emerging studies of growth factor injection to treat cartilage lesions have shown promising results. Studies have shown in vivo and in vitro, that various growth factors promote chondrocyte proliferation[41], chondrogenic differentiation of bone marrow-derived mesenchymal stem cells[42], and induce an upregulation of cartilage matrix derivatives[43]. Wu *et al* studied the effects of PRP used as a carrier for cultured autologous chondrocytes, leading to cartilage formation when implanted in the subcutaneous tissue of rabbit models[44].

Bone Injuries

Platelets have been found to be a natural vehicle for growth factors and cytokines that promote the process of bone mineralisation. Growth factors such as PDGF, IGF, and TGF-B are important modulators of this process[45,46]. Members of the TGF-B superfamily, together with bone morphogenic proteins released from mesenchymal stem cells, are responsible for triggering chondroblastic and osteoblastic differentiation and new bone matrix production[47].

An example of an animal study include one by Simman *et al* that showed accelerated fracture healing of rat femurs by PRP in a case-control study[48]. At 4 weeks of fracture healing, there was higher callus to cortex width ratio radiographically, along with increased bone strength and histological evidence of enhanced bone formation in the PRP group.

CLINICAL APPLICATIONS IN SPORTS MEDICINE

Tendon and Ligament injuries

Sanchez *et al*[5] combined open repair of completely torn Achilles tendons with application of an autologous PRGF in a case-control study. Although it was a retrospective study and subject numbers were small, Sanchez's study was one of the first to investigate the treatment of tendon injury with growth factors and had good results in terms of enhanced healing and functional recovery. In addition, there were no wound complications observed. Further studies of growth factor augmentation have also demonstrated faster recovery times for athletes with Achilles tendon and rotator cuff tears[28,49]. To lend further strength to the current literature of retrospective studies, several prospective randomised trials are underway to delineate the effects of growth factors.

In chronic elbow tendinosis treated with growth factor injection, Mishra *et al* found high rates (above 90%) of pain reduction, return to work and to daily activities at long term follow up of patients[17]. Similar to using growth factors, Connell *et al* assessing the efficacy of autologous blood injection under sonographic guidance for the treatment of lateral epicondylitis demonstrated reduction in pain score (100% at 6 months), with significant sonographic reduction in tendon thickness and hypoechoic changes seen[37]. Moon *et al* studied the injection of iliac bone marrow plasma after arthroscopic debridement in severe elbow tendinosis. In this observational follow-up study of 26 patients, improvement in pain scores and early recovery of daily activities were demonstrated[50].

PRGF has been used to enhance tendon grafts for ligament reconstructions. Sanchez and collaborators, in their study investigating the application of PRGF during anterior cruciate ligament (ACL) surgery showed that it influences the histologic characteristics of such grafts resulting in more remodelling compared with untreated grafts[36]. The influence of growth factors on the ligament was demonstrated by a number of animal studies. Murray *et al* have demonstrated improved healing in the primary intraoperative repair of porcine ACL, after biologic augmentation with growth factors[8]. While Kobayashi *et al* observed increased filling and vascularity around a central defect in a canine ACL model, after implantation with a Fibroblast Growth Factor pellet[51].

There is an ongoing randomised controlled multi-centre trial using PRP to treat plantar fasciitis by Peerbooms *et al* in the Netherlands[52]. This trial will compare treatment for chronic plantar fasciitis with a steroid injection versus an injection of autologous platelets. The authors will be assessing main outcome measures of pain relief and function up to 1 year after treatment.

Muscle Injuries

A muscle strain typically puts athletes out of action for several weeks or months and this can result in athletes missing a significant part of the

season[53]. Thus, affording a faster healing time and return to sport would spell tremendous benefits to athletes and teams. There are no randomised controlled trials involving human subjects, with only case series and pilot studies reported thus far. An unpublished case series by Cugat *et al* has been reported in the literature[27]. This was a case series of 14 professional soccer and basketball players who presented with 16 muscular injuries. A preparation of autologous PRP was injected, via ultrasound guidance, into each muscle tear after aspiration of the haematoma. Cugat *et al* reported an improvement in return-to-play interval, which was compared to previously published expected return-to-play data.

A pilot study found a significantly accelerated recovery time when professional athletes with muscle strains were injected with autologous conditioned serum[25]. The control group in this pilot study, however, was a retrospective analysis of previous athletes treated with conventional injection of anti-inflammatories or deproteinised dialysate.

While the improved time to return-to-play would seem like an enticing reason to consider growth factor injections for muscular injury, other researchers have advocated caution. This is due to concerns that a fibrotic response can be induced by TGF, released by the platelets' alpha granules[54]. This fibrotic healing can lead to an increased incidence of reinjury.

Chondral Injuries

Sanchez *et al* described a case report of the use of PRGF for a young soccer player who sustained a large, non-traumatic avulsion of the articular cartilage of the knee. In this approach, an intra-articular injection of PRGF was given after arthroscopic reattachment of the loose chondral body in the medial femoral condyle. Significant accelerated healing and functional recovery was observed, with the adolescent rapidly returning to activity symptom-free[6].

In the treatment of severe chondropathies of the knee, a pilot study by Kon *et al* of 100 consecutive patients with chronic degenerative knees demonstrated short-term symptomatic relief with intra-articular PRP injections. The study demonstrated that PRP had more beneficial effects than hyaluronic acid injections in reducing pain, symptoms and recovering articular function in patients[55].

Bone Injuries

Gandhi *et al*'s study of the use of PRP in treating fractures of the foot and ankle, found radiographic fracture union in revision operations for nonunions where PRP was applied[56]. These fracture nonunions had demonstrated no trace of PDGF and TGF-B. But Sanchez *et al* had mixed results when they applied PRP to clinical supracondylar and diaphyseal non-unions[57]. Other studies of bone healing in spinal fusion[58] and in distraction osteogenesis for limb lengthening[59] also show mixed results and are plagued by small sample size and not being randomised.

ISSUES WITH DOPING IN SPORTS

The use of growth factors is prohibited under the World Anti-Doping Agency (WADA) Prohibited List 2010 under section S2[60]. Under the same section, platelet-derived preparations like PRP, when delivered intramuscularly, are prohibited. Other routes of delivery require a declaration of use. The use of autologous blood product as a means to deliver growth factors is prohibited under section M1 under blood doping. A doping violation encompasses the use of "any other growth factor affecting muscle, tendon, or ligament protein synthesis… vascularisation… [or] regenerative capacity…"[60]. If the use of growth factors or the use of any of the aforementioned prohibited methods were indicated in the treatment of clinical conditions in an athlete, application for a therapeutic use exemption would then be required.

However, things will change in 2011 as platelet-derived preparations, referred to as PRP or blood spinning by WADA, have been removed from the Prohibited List for 2011[61]. This is based on WADA's assertion that current clinical studies show that platelet-derived preparations "do not demonstrate a potential for performance enhancement beyond a potential therapeutic effect…"[61]. This comes as little surprise in the ongoing debate over PRGF and doping as researchers have previously argued that growth factor preparations are unlikely to present any performance-enhancing properties to athletes as growth factors have a short half-life and are present in amounts that cannot produce significant systemic anabolic effects[16]. The particular isoform of growth factor in PRGF is not the isoform responsible for muscular hypertrophy.

Individual growth factors are still prohibited when given separately as purified substances.

The International Olympic Committee (IOC) acknowledges the fine line that exists between using PRGF for therapy and the potential for illegal performance enhancement. Thus, an IOC Consensus Statement in 2008 advocated an improved understanding of the molecular basis of the effects of growth factors, to ensure the optimization of therapies using PRGF and minimise the potential risks to patients and athletes[62].

POTENTIAL ADVANTAGES AND LIMITATIONS OF PRGF

Advantages of PRGF

The use of PRGF preparations in sports-related injuries presents several advantages. The risk of rejection of the injected PRGF is low as the preparation is derived from the patient's own blood[27]. Secondly, the procedure for preparing the PRGF is simple and fast, allowing for treatment to be administered on the same day and in a rapid way[27].

Limitations of PRGF

Excessive fibrosis has been raised by researchers as a potential local complication of growth factor administration. Fibrosis occurs physiologically at the last phase of muscle healing, and a growth factors play a key role in regulating the balance between regeneration and fibrosis[54]. The use of a single-dose of growth factors injected at concentrations higher than normal can result in excessive fibrosis, inhibiting muscle regeneration[63,64]. There is also a theoretical risk that the application of PRGF can result in carcinogenesis or a cancer-like effect, mediated by the enhanced proliferation and migration of mesenchymal cells. However, researchers argue against the risk of cancer as growth factors do not enter these cells and do not cause DNA mutation[16].

There is a lack of standardisation in the harvesting of PRGF in the current literature, causing problems with data interpretation and inconsistent results between studies[27]. Standardisation of PRGF production protocols can ensure a consistent and reliable quantification of the concentrations of growth factors administered in future trials. Applying these protocols in a randomised controlled trial would yield high level evidence to determine the efficacy of growth factors.

CONCLUSION

Growth factors delivered by autologous preparations of platelet-rich or conditioned plasma have been shown to yield a significant beneficial effect on the healing of ligaments, muscle, tendon and chondral injuries. While current animal and human studies have shown good early results in terms of earlier functional recovery and return to sport in athletes, further research on PRGF harvesting as well as delivery protocols is needed. These treatments will only be effective if the underlying causative factors and biomechanical errors are corrected. Physicians should still employ and exhaust standard therapy and treatment before considering PRGFs.

REFERENCES

1. Boyer MI, Watson J, Lou J, Manske PR, Gelberman RH, Cai SR. Quantitative variation in vascular endothelial growth factor mRNA expression during early flexor tendon healing: an investigation in a canine model. J Orthop Res. 2001;19(5):869–72.
2. Dahlgren LA, Mohammed HO, Nixon AJ. Temporal expression of growth factors and matrix molecules in healing tendon lesions. J Orthop Res. 2005;23(1):84–92.
3. Menetrey J, Kasemkijwattana C, Day CS, Bosch P, Vogt M, Fu FH, et al. Growth factors improve muscle healing in vivo. J Bone Joint Surg Br. 2000;82(1):131-7.
4. Molloy T, Wang Y, Murrell GAC. The roles of growth factors in tendon and ligament healing. Sports Med. 2003;33(5):381–94.
5. Sanchez M, Anitua E, Azofra J, Andia I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med. 2007;35(2):245–51. Epub 2006 Nov 12.
6. Sanchez M, Azofra J, Anitua E, Andia I, Padilla S, Santisteban J, et al. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. Med Sci Sports Exerc. 2003;35(10):1648–52.
7. Sanchez M, Azofra J, Aizpurua B, Elorriaga R, Anitua E, Andia I. Use of autologous plasma rich in growth factors in arthroscopic surgery. Cuadernos de Artroscopia. 2003;10:12-19.
8. Murray MM, Spindler KP, Ballard P, Welch TP, Zurakowski D, Nanney LB. Enhanced histologic repair in a central wound in the ACL with a collagen-platelet-rich plasma scaffold. J Orthop Res. 2007;25(8):1007–17.
9. Epply BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet rich plasma: implications for wound healing. Plast Reconstr Surg. 2004;114(6):1502-8.
10. Chang J, Thunder R, Most D, Longaker MT, Lineaweaver WC. Studies in flexor tendon wound healing: neutralizing antibody to TGF-B1 increases postoperative range of motion. Plast Reconstr Surg. 2000; 105(1):148–55.
11. Marui T, Niyibizi C, Georgescu HI, Cao M, Kavalkovich KW, Levine RE, et al. Effect of growth factors on matrix synthesis by ligament fibroblasts. J Orthop Res. 1997;15(1):18-23.
12. Bennett NT, Schultz G. Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am J Surg. 1993; 65(6):728–37.
13. Lynch SE, Colvin R, Antoniades HN. Growth factors in healing: single and synergistic effects on partial thickness porcine skin wounds. J Clin Invest. 1989;84(2):640–6.

14. Abrahamsson SO. Similar effects of recombinant human insulin-like growth factor-I and II on cellular activities in flexor tendons of young rabbits: experimental studies in vitro. J Orthop Res. 1997;15(2):256–62.
15. Chan BP, Fu S, Qin L, Rolf CG, Chan K. Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: a rat patellar tendon model. Acta Orthop Scand. 2000;71(5):513–8.
16. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. Br J Sports Med. 2008;42(5):314–20. Epub 2007 Nov 5.
17. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med. 2006;34(11):1774–8.
18. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surgery. 2004;62(4):489–96.
19. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: optimizing the healing environment. Arthroscopy. 2010;26(2):269–78.
20. Anitua E, Sanchez M, Nurden AT, Nurden P, Orive G, Andia I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol. 2006;24(5):227–34. Epub 2006 Mar 15.
21. Tidball JG. Inflammatory cell response to acute muscle injury. Med Sci Sports Exerc. 1995;27(7):1022–32.
22. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone. 2004;34(4):665–71.
23. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004;91(1):4-15.
24. Asfaha S, Cenac N, Houle S, Altier C, Papez MD, Nguyen C, et al. Protease-activated receptor-4: A novel mechanism of inflammatory pain modulation. Br J Pharmacol. 2007;150(2):176-85. Epub 2006 Dec 18.
25. Wright-Carpenter T, Klein P, Schaferhoff P, Appell HJ, Mir LM, Wehling P. Treatment of muscle injuries by local administration of autologous conditioned serum: a pilot study on sportsmen with muscle strains. Int J Sports Med. 2004;25(8):588–93.
26. Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in the whole blood by physico-chemical induction. Inflamm Res. 2003;52(10):404-7.
27. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med. 2009;37(11):2259–72.
28. Anitua E, Sanchez M, Nurden AT, Zalduendo M, de la Fuente M, Azofra J, et al. Reciprocal actions of platelet-secreted TGF-beta1 on the production of VEGF and HGF by human tendon cells. Plast Reconstr Surg. 2007;119(3):950–9.
29. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg Am. 2005;87(1): 187–202.
30. Woodall JR, Tucci M, Mishra A, Benghuzzi H. Cellular effects of platelet rich plasma: a study on HL-60 macrophage-like cells. Biomed Sci Instrum. 2007;43:266–71.
31. Woodall J Jr, Tucci M, Mishra A, Asfour A, Benghuzzi H. Cellular effects of platelet rich plasmainterleukin-1 release from platelet rich plasma treated macrophage cells. Biomed Sci Instrum. 2008;44:489–94.
32. Kawamura S, Ying L, Kim HJ, Dynybil C, Rodeo SA. Macrophages accumulate in the early phase of tendon-bone healing. J Orthop Res. 2005;23(6):1425–32
33. Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res. 2007;25(2):230–40.
34. Kajikawa Y, Morihara T, Sakamoto H, Matsuda K, Oshima Y, Yoshida A, et al. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. J Cell Physiol. 2008;215(3):837–45.
35. Gulotta LV, Rodeo SA. Growth factors for rotator cuff repair. Clin Sports Med. 2009 Jan;28(1):13–23.
36. Sánchez M, Anitua E, Azofra J, Prado R, Muruzabal F, Andia I. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. Arthroscopy. 2010 Apr;26(4):470–80.
37. Connell DA, Ali KE, Ahmad M, Lambert S, Corbett S, Curtis M. Ultrasound-guided autologous blood injection for tennis elbow. Skeletal Radiol. 2006 Jun;35(6):371–7.
38. Kasemkijwattana C, Menetrey J, Bosch P, Somogyi G, Moreland MS, Fu FH, et al. Use of growth factors to improve muscle healing after strain injury. Clin Orthop Relat Res. 2000;(370):272–85.
39. Musaro A, McCullagh KJ, Paul A, Houghton L, Dobrowolny G, Molinaro M, et al. Localized IGF-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet. 2001;27(2):195–200.
40. Hammond JW, Hinton RY, Curl LA, Muriel JM, Lovering RM. Use of autologous platelet-rich plasma to treat muscle strain injuries. Am J Sports Med. 2009;37(6):1135-42. Epub 2009 Mar 12.
41. Tay AG, Farhadi J, Suetterlin R. Cell yield, proliferation, and postexpansion differentiation capacity of human ear, nasal, and rib chondrocytes. Tissue Eng. 2004;10(5-6):762–70.
42. Mizuta H, Kudo S, Nakamura E, Otsuka Y, Takagi K, Hiraki Y. Active proliferation of mesenchymal cells prior to the chondrogenic repair response in rabbit full-thickness defects of articular cartilage. Osteoarthritis Cartilage. 2004;12(7):586–96.
43. Akeda K, An HS, Okuma M, Attawia M, Miyamoto K, Thonar EJM, et al. Platelet-rich plasma stimulates porcine articular chondrocyets and matrix biosynthesis. Osteoarthritis Cartilage. 2006;14(12):1272–80
44. Wu W, Chen F, Liu Y, Ma Q, Mao T. Autologous injectable tissue-engineered cartilage by using platelet-rich plasma: experimental study in a rabbit model. J Oral Maxillofac Surg. 2007;65(10):1951–7.
45. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants. 1999;14(4):529-35.
46. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(6):638-46.
47. Roldán JC, Jepsen S, Miller J, Freitag S, Rueger DC, Açil Y, et al. Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7. Bone. 2004 Jan;34(1):80-90.
48. Simman R, Hoffmann A, Bohinc RJ, Peterson WC, Russ AJ. Role of platelet-rich plasma in acceleration of bone fracture healing. Ann Plast Surg. 2008;61(3):337–44.
49. Randelli PS, Arrigoni P, Cabitza P, Volpi P, Maffulli N. Autologous platelet rich plasma for arthroscopic rotator cuff repair: a pilot study. Disabil Rehabil. 2008;30(20-22):1584–9.
50. Moon YL, Jo SH, Song CH, Park G, Lee HJ, Jang SJ. Autologous bone marrow plasma injection after arthroscopic debridement for elbow tendinosis. Ann Acad Med Singapore. 2008;37(7):559-63.
51. Kobayashi D, Kurosaka M, Yoshiya S, Mizuno K. Effect of basic fibroblast growth factor on the healing of defects in

the canine anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc. 1997;5(3):189-94.
52. Peerbooms JC, van Laar W, Faber F, Schuller HM, van der Hoeven H, Gosens T. Use of platelet rich plasma to treat plantar fasciitis: design of a multi centre randomized controlled trial. BMC Musculoskelet Disord. 2010;11:69.
53. Junge A, Dvorak J, Graf-Baumann T. Football injuries during World Cup 2002. Am J Sports Med 2004;32(1 Suppl):23S-27S.
54. Chan YS, Li Y, Foster W, Fu FH, Huard J. The use of suramin, an antifibrotic agent to improve muscle recovery after strain injury. AAm J Sports Med. 2005;33(1):43-51.
55. Kon E, Buda R, Filardo G, Di Martino A, Timoncini A, Cenacchi A, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):472-9. Epub 2009 Oct 17.
56. Gandhi A, Bibbo C, Pinzar M, Lin SS. The role of platelet-rich plasma in foot and ankle surgery. Foot Ankle Clin. 2005;10(4):621–37, viii.
57. Sanchez M, Anitua E, Cugat R, Azofra J, Guadilla J, Seijas R, et al. Nonunions treated with autologous preparation rich in growth factors. J Orthop Trauma. 2009;23(1):52–9.
58. Carreon LY, Glassman SD, Anekstein Y, Puno RM. Platelet gel(AGF) fails to increase fusion rates in instrumental posterolateral fusions. Spine (Phila Pa 1976). 2005 May 1;30(9):E243-6; discussion E247.
59. Kitoh H, Kitakoji T, Tsuchiya H, Katoh M, Ishiguro N. Transplantation of culture expanded bone marrow cells and platelet rich plasma in distraction osteogenesis of the long bones. Bone. 2007;40(2):522–8.
60. The World Anti-Doping Agency. The 2010 Prohibited List; International Standard [Internet]. 2009 Sep 19 [cited 2010 Jun 1]. 9 p. Available from: http://www.wada-ama.org/rtecontent/document/2010_Prohibited_List_FINAL_EN_Web.pdf.
61. The World Anti-Doping Agency. The 2011 Prohibited List; International Standard [Internet]. 2010 Sep 18 [cited 2010 Nov 1]. Available from: http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/To_be_effective/WADA_Prohibited_List_2011_EN.pdf.
62. Ljungqvist A, Schwellnus MP, Bachl N, Collins M, Cook J, Khan KM, et al. International Olympic Committee consensus statement: molecular basis of connective tissue and muscle injuries in sport. Clin Sports Med. 2008;27(1):231-9, x-xi.
63. Shen W, Prisk V, Li Y, Foster W, Huard J. Inhibited skeletal muscle healing in cyclooxygenase-2 gene-deficient mice: the role of PGE2 and PGF2alpha. J Appl Physiol. 2006;101(4):1215–21. Epub 2006 Jun 15.
64. Prisk V, Huard J. Muscle injuries and repair: the role of prostaglandins and inflammation. Histol Histopathol. 2003;18(4):1243–56.